CXXXIX. OBSERVATIONS ON THE EFFECT OF VARIOUS CARBOHYDRATES ON THE KETOSIS OF STARVATION IN HUMAN SUBJECTS.

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It is well known that in man after a relatively short period of starvation, or during a period of diet very low or entirely deficient in carbohydrate, a considerable ketosis appears. The time of onset of a positive nitroprusside reaction in the urine depends upon the composition of the diet prior to the commencement of starvation. A previous diet high in carbohydrate will postpone the ketosis whilst one high in fat and protein and low in carbohydrate will bring it on much more rapidly. Since during starvation the organism is thrown back upon its own tissues, it follows that what is going to happen will depend upon the composition of these tissues. Hence in fat subjects we expect a more rapid onset of ketosis than in thin subjects. This is well illustrated in the case of a starving fat woman investigated by Folin and Denis [1915]. This woman excreted after one day's fasting 270 mg. of acetoacetic acid and in succeeding days very large quantities of acetoacetic and hydroxybutyric acids. The professional faster Succi, who, according to Brugsch's description [1905], seems to have been a man of average build, produced much smaller quantities of the ketonic substances than the fat woman, even after very much longer periods of starvation. In general a man of medium build will not show a positive nitroprusside reaction in the urine in less than 30 hours of complete starvation (no food or drink). In our experience of a considerable number of fasts the time varied from 20 hours after a previous diet low in carbohydrate to 36 hours after an average mixed diet.

The cause of the ketosis seems to depend principally on carbohydrate deficiency. Once a certain minimum of carbohydrate is reached further depletion finds expression in ketosis.

As a result of careful observation of the time of onset of a recognisable ketosis in starving normal subjects, we have received the impression that the onset of acetonuria is essentially an acute phenomenon. If the urine of a starving subject be examined after the first 24 hours' starvation at hourly intervals for 5 or 6 hours, and then every 15 minutes, one suddenly reaches a urine giving a marked nitroprusside reaction. Quantitative estimations of

ketonic substances in two consecutive urines separated by a 15 minutes' interval, one being negative and the other positive to the nitroprusside test, show a difference of from perhaps 3 or 4 mg. % in the negative to as much as 20 to 30 mg. % in the positive.

Another factor which influences the time of onset of ketosis is the fluid intake. Ehrström [1922] draws attention to the influence of water hunger on the excretion of the ketonic acids. He cites the case of a middle-aged man who had vomited everything for 3 days and then developed a strong acetonuria and acetone-charged breath. Both these symptoms diminished in intensity when, without any food being given, an infusion of saline was carried out. He also refers to a similar experience with a case of pyloric stenosis. The polyuria of diabetes is also regarded by him as a contributory factor in the ketogenesis. He refers also to the acetonuria of dipsophobes.

We have not as yet tested this point fully but up to the present we can say that starvation ketosis appears more rapidly in warm weather and much more rapidly if no fluid is taken. It is, however, not to be supposed that there is any serious shortage in water in so short a period as two days. The body undoubtedly has considerable stores of water upon which it can draw before serious symptoms are produced. It is of interest to recall that the metabolism of 100 g. fat produces about 105 g. water whilst 100 g. protein give rise to about 40 g. water.

In the experiments to be described a considerable number of fastings were carried out and in most of them no fluid was taken for 36 hours. Such a fast can bring about a good deal of disturbance and in one case (Dr A. L.) the ketosis was so intense that some water had to be given, after which the condition was much improved. In general, however, an average man can starve for 36 hours even without fluid, with very little upset. Certain symptoms, however, frequently appear, as tendency to headaches, fatigue, difficulty in concentration and cramping pains in the muscles of the extremities. On one occasion an intense constricting pain was felt in the chest and this was followed by a sudden vertigo and fainting attack. It was found that the blood sugar at the time of these symptoms was down to 0·056 %. The cramping pains are probably of the same nature as those felt by cases of severe diarrhoea, with frequent large loss of fluid—the extreme cases being found in cholera. In spite of these symptoms, it was decided in our experiments to undergo the starvation without fluid in order to develop as considerable a ketosis as possible in as short a time as possible.

The ketosis of starvation differs in certain ways from that occurring in diabetics. Whereas in starvation the ketosis increases, in diabetics fasting brings about a marked diminution in the excretion of the ketonic substances. At first sight this would suggest a difference in mechanism in the two cases, a supposition which Joslin [1917] supports.

It seems, however, more reasonable to believe that the diminution in ketosis following starvation in the diabetic is due simply to the withdrawal

of his high fat diet and in the normal to the removal of the relatively high carbohydrate diet. This view is taken by Labbé *et al.* [1921], Shaffer [1921] and Woodyatt [1916].

Although starvation will reduce ketosis in the diabetic it will not remove it entirely.

In both diabetic and starvation ketosis there is a deficiency in carbohydrate metabolism; in the former due to failure of the insulin mechanism, in the latter due to withdrawal of carbohydrate.

The analogy between the condition in starvation and that in diabetes can be extended further to include diminished tolerance to carbohydrate, low respiratory quotient, as well as ketosis. These facts will appear in the data presented below.

Experimental.

1. The plan of experiment was to undergo a preliminary period of complete starvation and then, having determined the fasting levels of R.Q., blood sugar and excretion of ketonic substances, to ingest solutions of the various sugars and make similar observations.

Having regard to the different constitution of the sugars and to the different degrees of storage and oxidation which have been observed after their ingestion, it was considered probable that they would show *in vivo* different antiketogenic powers.

Owing to the different solubilities of the sugars the degree of absorption from the gut is different for each sugar. Ordinarily sugar does not appear in the faeces to any considerable extent but it is certain that decomposition takes place in the intestine as a result of the activity of the intestinal flora.

2. To detect small quantities of acetoacetic acid we used the nitroprusside reaction.

3. To estimate the ketonic substances we used a method described by the writer elsewhere [1925].

4. Blood sugars were determined by MacLean's method.

5. For the determination of respiratory quotients Haldane's apparatus with the Douglas bag was used. Although a certain amount of movement is necessary in order to collect blood and urine, it is possible with some practice to carry this out without interfering appreciably with the R.Q.

6. The substances investigated were: glucose, fructose, mannose, sucrose, lactose, maltose, galactose and glycerol.

7. Except where otherwise stated the subject of experiment was the writer.

Exp. 1. In this experiment the sugar examined was pure *glucose.* A fast was commenced at 7 p.m. 30. xii. 24. The urine from 8 p.m. 31. xii. 24 till 9. a.m. 1. i. 25 was collected.

At 10.30 a.m. 1. i. 25 the fasting blood sugar and respiratory quotient were determined. At 11.20 a.m. 50 g. pure glucose in 150 cc. water were taken.

The findings are given in Table I.

Table I.

Period of starvation, 40½ hrs.

Time a.m.	Urine cc.	Nitro-prus-side test	Acetoacetic acid + acetone mg. (acetone) per cc.	Total	per hr.	β-Hydroxybutyric acid mg. per cc.	Total	per hr.	Blood sugar %	R.Q.	Fehl-ing's test
9.0	215	+	0·134	28·8	2·2	0·88	189·2	14·6	.	.	–
10.30	23	+	0·105	2·4	1·6	0·93	21·4	14·3	0·113	0·73	–
10.45	3	+	.	.	.	.	.	.	.	.	.
11.15	14·5	+	0·197	2·9	5·7	0·567	8·2	16·4	.	.	–
11.20	50 g. glucose taken										
11.30	11	+	0·153	1·7	6·7	0·542	5·96	23·9	.	.	.
11.45	8	+	0·192	1·5	6·1	0·876	6·81	27·2	0·104	0·74	–
12.0 p.m.	10·5	+	.	.	.	.	.	.	0·184	.	.
12.15	8·5	+	0·163	1·4	5·6	0·365	3·1	12·4	0·189	0·75	+
12.30	6·5	–	0·039	0·25	1·01	0·869	5·7	22·8	0·187	.	+
12.45	8·0	–	.	.	.	.	.	.	0·189	.	+
1.5	8·0	–	0·041	0·33	1·18	0·806	6·45	19·34	0·141	0·81	–
1.30	14·0	–	.	.	.	.	.	.	.	.	–
2.55	25	–	.	.	.	.	.	.	0·099	.	–
3.45	10	+	0·192	1·92	2·3	1·08	10·8	12·9	.	.	–

From this table it will be seen that

1. The nitroprusside reaction became negative 1 hour after the ingestion of the glucose and remained negative for a further 3¼ hours. The excretion of acetoacetic acid and acetone fell from about 6 mg. per hour to negligible quantities and then began to rise with the re-establishment of the nitroprusside reaction. The figures given for acetoacetic acid during the period of negative nitroprusside reaction can only be regarded as approximations, as the method of estimation was not sensitive for quantities below 0·1 mg. The actual content in acetoacetic acid during this period was probably lower than the figures given.

2. The excretion of β-hydroxybutyric acid throughout this experiment showed no tendency to fall, even during the period of negative nitroprusside reactions.

3. The blood sugar curve was definitely abnormal and the glycosuria which occurred never normally occurs in the writer after 50 g. glucose.

For comparison we give the figures obtained after 50 g. glucose under ordinary post-absorptive conditions.

Normal blood sugar curve following 50 g. glucose taken 3 hours after morning meal.

Time	Blood sugar %	Fehling's test on urine
11.41 a.m.	0·122	–
11.50 50 g. glucose taken		
11.55	0·129	–
12.15 p.m.	0·159	–
12.35	0·150	–
12.55	0·125	–
1.15	0·117	–

4. The fasting respiratory quotient was 0·73 and after the glucose it rose slowly to 0·81 in 1½ hours. Normally the rise following 50 g. glucose is

to 0·9 in 1 hour. It will further be observed that the rise in R.Q. occurred after the maximum blood sugar had been passed.

5. The period of negative nitroprusside reaction corresponded to that of the fall from the maximum blood sugar to the fasting level.

Exps. 2 *and* 3. These were carried out on two normal young men (Dr A. L. and Dr A. C.) who kindly volunteered to undergo the rather trying experiment. As these experiments were exactly similar to Exp. 1 it will only be necessary to summarise the findings.

Table II.

T_1 = Time to overcome existing ketosis.
T_2 = Time during which the keto-antiketogenic balance was preserved.

Exp.	Subject and period of starvation	Glucose g.	T_1 hours	T_2 hours	Blood sugar curve	Max. R.Q.
1	M.W.G. 40½ hrs.	50	1	3½	Abnormal	0·81
2	A.L. 38	75	2½	1¾	,,	0·86
3	A.C. 40	50	1	3	,,	0·82

By abnormal blood sugar curve is meant a curve indicating a diminished tolerance to the particular sugar administered.

The followed facts were elicited from these experiments:

1. Glucose is very efficient as an antiketogenic agent, but the rapidity of control of a starvation ketosis varies in different subjects. As a result of several starvations and ingestion of 50 g. glucose after 40 hours the values of T_1 and T_2 were found to be remarkably constant at 1 and 3 hours, respectively, in the case of the writer. It was therefore considered that a basis for qualitative comparison of the antiketogenic values of the sugars was provided by such experiments in the same subjects.

2. The tolerance to glucose after starvation is markedly diminished. The blood sugar curve after starvation always shows an exaggerated rise and much delayed return to normal levels. Glycosuria commonly occurred after quantities of glucose well below the normal tolerance.

3. In each case the maximum R.Q. was such as to indicate deficient oxidation, and this value was attained in each experiment some time after the maximum blood sugar was reached.

4. The period of active antiketosis (negative nitroprusside test in urine) always corresponded to the period of fall in blood sugar.

The abnormalities in blood sugar curves following starvation and diets low in carbohydrate have been recorded by several observers. Kageura [1922] found that the hyperglycaemia in man following the ingestion of the Japanese test meal (100 g. rice + 2 eggs) was greater after a carbohydrate-poor diet than after a diet rich in carbohydrate. In some cases 100 g. rice produced glycosuria after a meat-fat period. He found that protein and fat were equally effective in lowering carbohydrate tolerance.

Greenwald [1924], however, working with dogs, found that a diet of protein does not lower carbohydrate tolerance to the same extent that a fat diet does.

This is comprehensible when we consider that 56 % of protein is potential carbohydrate whilst only 10 % of fat is capable theoretically of yielding sugar, though actually we have never detected a perceptible rise in blood sugar after administration of glycerol to a normal subject.

Southwood [1923] also found that there was marked diminution in carbohydrate tolerance after starvation and low carbohydrate diet. Injection of a small dose of insulin produced a readjustment of the blood sugar curve to almost normal.

Brailsford Robertson, commenting on Southwood's paper, takes the view that during starvation, and hence low blood sugar, there is diminished activity of the islet tissue in the pancreas and hence physiological rest to this tissue. This he considers to be the rationale of Allen's fasting treatment of diabetes.

This indeed seems very probable, particularly as the R.Q. following 50 g. glucose and even 75 g. glucose in none of our cases rose to 0·9 or over. This would certainly indicate diminished power of oxidation which evidently we must refer back to a diminished secretion of insulin.

Having regard to the modern conception proposed by MacLean and de Wesselow [1920] that a rise in blood sugar stimulates the glycogenic mechanism, it seemed probable that a period of starvation would bring about a diminution in storage power. This conception of hyperactivity of the glycogenic function was based on the fact that after a dose of carbohydrate the blood sugar curve in its fall often reached values below the fasting level. But it must be remembered that this does not always occur in normal conditions. After starvation we have not, in general, observed a fall below the fasting level.

The next sugar investigated was *fructose*. Two experiments were made exactly similar to those with glucose. In the second of these 50 g. glucose were ingested after the re-establishment of the nitroprusside test in the urine, in order to see if the absorption of the fructose had readjusted the disturbed carbohydrate metabolism. We tabulate the results as before.

Table III.

Exp.	Period of starvation	Fructose g.	T_1 hours	T_2 hours	Blood sugar curve	Max. R.Q.
1	39 hrs.	50	1¾	1	Rose to 0·133	0·77
2	39	50	1½	1	,, 0·120	0·77
		followed by 50 g. glucose	1	3	Normal	—

The average normal fructose curve for the writer is given for comparison, the sugar having been ingested at the time of the first blood sugar determination.

Time (mins.)	0	30	60	90	120
Blood sugar %	0·110	0·130	0·120	0·105	0·101

We must therefore consider that the fructose curve after starvation is not appreciably changed from the normal. If we accept the view that the fructose curve is an index of the carbohydrate-storing power of the organism, then it

would seem that after starvation storage power is normal. Unfortunately the fructose test is not unequivocal, but it would seem that deficient storage is not the principal cause of the lowered carbohydrate tolerance after starvation.

The results with fructose may be summarised as follows:

1. Fructose acts very efficiently as an antiketogenic agent, having overcome the ketosis in about 1½ hours and preserved a balance for 1 hour.

2. The period of negative nitroprusside reaction in the urine corresponded with the period of fall in blood sugar, showing that active antiketogenesis takes place during storage and oxidation of the carbohydrate.

3. The blood sugar curve did not indicate any deficiency in storage power, and normal carbohydrate metabolism was re-established by the fructose as shown by a normal glucose curve after the fructose curve had returned to normal.

4. The respiratory quotients indicated deficient oxidation and the maximum R.Q. was reached some time after the maximum blood sugar was reached. Bornstein and Holm [1922] have also shown that there is a latent period between the administration of sugar and the rise in R.Q., which contrasts with the immediate rise in blood sugar. It would seem that a certain height of blood sugar must be reached before the mechanism of oxidation is brought into full play. When the requisite level is reached the insulin secretion is stimulated and oxidation proceeds rapidly.

The next sugar investigated was *galactose*. A preliminary starvation of 42 hours was undergone, at the end of which a considerable ketosis had appeared. As in the former experiments 50 g. of the sugar in 200 cc. water were taken.

The findings in this experiment may be summarised as follows:

1. The ketosis was not affected by the ingestion of galactose. The nitroprusside reaction remained positive and the quantitative results agreed with the qualitative findings, except for a tendency to a diminution in the hourly excretion; but complete keto-antiketogenic balance never occurred.

That the galactose was absorbed appears in the fact that a powerful galactosuria and a rise in blood sugar occurred during the experiment.

It would appear that this sugar is incapable, at least during the period of this experiment, of controlling the ketosis of starvation.

2. The blood sugar curve commenced at 0·08 %, rose in 1 hour to 0·135 % and regained the normal in a further 80 minutes.

3. The R.Q. rose from a fasting level of 0·704 to 0·736 an hour after the galactose was taken and fell again to 0·704 in another hour.

4. The urine showed a powerful reduction of Fehling's solution when the blood sugar was between 0·104 % and 0·135 %, and continued to do so for nearly 4 hours, long after the blood sugar had returned to normal.

5. 50 g. glucose administered after the galactose blood sugar curve had returned to normal, produced an entirely normal curve, showing that the galactose had readjusted the disturbed carbohydrate metabolism.

6. There is a diminution in storage and oxidation of this sugar after starvation.

Normally 50 g. galactose taken by the writer 3 hours after a meal produces practically no rise in blood sugar, but galactosuria occurs in less than 30 minutes after taking the sugar.

As a result of many experiments on normal subjects with 50 g. galactose we have concluded that it gives rise to a blood sugar curve comparable to that of fructose, has no definite threshold and filters through the kidney with ease.

A similar experiment was carried out with *lactose* after a preliminary starvation of 40 hours. The results were very similar to those obtained with galactose. The ketosis was unaffected by 50 g. lactose and even the administration of a further 50 g. 3 hours after the first dose failed to overcome the starvation ketosis.

There was some doubt whether the lactose was completely absorbed as a considerable diarrhoea occurred later, and 15 estimations of blood sugar at intervals of about 15 minutes showed no appreciable changes. Lactosuria, however, occurred 30 minutes after the first dose of lactose and continued throughout the experiment; some of the sugar, therefore, was certainly absorbed.

The question as to the normal tolerance to lactose is unsettled. Folin and Berglund [1922] consider that it has no threshold, since they obtained lactosuria after 30 g. lactose without any attendant rise in blood sugar. Others, however, have found no lactosuria after more than 100 g. In our experiments we have in general found lactosuria after 50 g. and it has been present at low levels of blood sugar, *e.g.* 0·102 %, 0·096 %. We must, therefore, subscribe to the view that lactose has no real threshold and can appear in the urine at any level of blood sugar.

The next sugar examined was *sucrose*. The preliminary period of starvation was 44 hours, after which 50 g. sucrose in 200 cc. of water were taken. The ketosis developed was of the same order as those in former experiments and the conditions were as far as possible identical.

The results may be summarised as follows:

1. The sucrose rapidly and effectively controlled the ketosis. In 1 hr. 2 mins. the nitroprusside reaction became negative and remained so for a period of 1 hr. 7 mins. This sugar, therefore, whilst overcoming the ketosis as rapidly as glucose or fructose, was unable to preserve the keto-antiketogenic balance for more than 1 hr. 7 mins.

The normal curve for the writer is as follows:

Time	Blood sugar %	
Fasting	0·100	
50 g. sucrose taken		
30 mins. later	0·145	No glycosuria
60 „	0·164	
90 „	0·153	
120 „	0·090	

2. The blood sugar curve showed the abnormal rise and extended period of fall which has been observed with glucose. The fasting level was 0·09 % and 44 minutes after the sucrose was taken the blood sugar was 0·206 % and took 2 hrs. 40 mins. to return to 0·111 %.

3. The R.Q. showed a rise from 0·71 to 0·83 62 minutes after the sugar was taken and 18 minutes after the blood sugar had reached a maximum. It subsequently fell to 0·71.

4. The period of negative nitroprusside reaction corresponded to the period of fall in blood sugar.

5. Glycosuria occurred at a blood sugar level of 0·206 % and continued until the blood sugar was 0·11 %.

A similar experiment was carried out using *maltose*, the initial period of starvation being 37½ hours.

The findings may be summarised as follows:

1. 50 g. maltose rapidly and effectively controlled the ketosis. The nitroprusside reaction became negative in 1 hr. 8 mins. and remained negative for a further 2 hours.

2. The blood sugar starting at 0·097 % rose to a maximum of 0·179 % in 1 hr. 13 mins. and reached the fasting level in a further 90 mins.: 50 g. maltose normally raise the blood sugar to 0·16 % in 30 minutes and it reaches the normal again in a further 60 minutes.

The deficiency in carbohydrate tolerance after starvation is thus again manifest, although maltose does not exhibit it as well as glucose or sucrose. The urine throughout the experiment showed no tendency to reduce Fehling's solution.

3. The nitroprusside reaction remained negative throughout the period of fall in blood sugar.

The next sugar investigated was *mannose* which differs from glucose only in the arrangements of the H and OH groups at the second carbon atom. In taste it is peculiar. At first it seems quite sweet but in a moment or two a quinine-like flavour appears.

In the experiment the preliminary period of starvation was 39 hours. The mannose produced a violent intestinal disturbance with diarrhoea. The ketosis was entirely unaffected and the blood sugar showed no significant change. No reduction of copper occurred in the urine. It would appear that the mannose was not well absorbed so that we cannot at present be certain of its efficacy as an antiketogenic agent.

The last experiment deals with the effect of *glycerol* on the ketosis of starvation.

It is generally stated that glycerol is capable of forming carbohydrate in the body and that it increases the excretion of sugar when administered to diabetics; it has also been claimed that glycerol exerts some antiketogenic action. In man 20 g. glycerol can be completely oxidised. On taking 27 g. in one dose, 0·5 to 1 g. appears in the urine. It seemed, therefore, probable that glycerol would be effective in overcoming starvation ketosis.

To test this a fast of 38 hours was undergone after which 100 g. glycerol in 200 cc. water were taken. After taking this solution a violent headache was experienced which made it almost impossible to continue.

Summary of findings:

1. A very pronounced diuresis was observed, the volume passed every 15 minutes being about 40 cc. as compared with an average of 9 cc. in former experiments.

2. The nitroprusside test in the urine decreased somewhat in intensity but the total excretion of ketonic substances did not diminish.

3. The blood sugar increased from 0·09 to 0·113 % in the first half-hour after the glycerol was taken and returned to 0·09 % an hour later.

4. There was a marked excretion of glycerol in the urine.

We conclude from this experiment that glycerol has no antiketogenic action and, in doses of 100 g., produces no marked change in blood sugar.

SUMMARY.

1. The antiketogenic activity of certain sugars has been examined by administration during starvation ketosis.

2. Glucose, fructose, sucrose, and maltose have an antiketogenic action, whilst galactose, mannose, lactose and glycerol are inert.

3. The deficiency in carbohydrate tolerance after starvation, has been examined. Evidence of some deficiency in storage power and oxidation after starvation has been presented. Normal conditions are re-established after the administration of carbohydrate.

4. The rise in respiratory quotient following the ingestion of a sugar has been shown to occur some time after the maximum blood sugar is reached.

5. The maximum antiketogenic action of the sugar, as evidenced by a negative nitroprusside reaction, has been shown to occur during the period of fall in blood sugar, *i.e.* during storage and oxidation of the sugar.

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